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(21) International Application Number: PCT/US93/04070 (22) International Filing Date: 5 May 1993 (05.05.93) (30) Priority data: 878,608 5 May 1992 (05.05.92) US (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Box OTT, Bethesda, MD 20892 (US). (72) Inventors; and (75) Inventors/Applicants (for US only) : STERNBERG, Esther, M. [US/US]; 8816 Montgomery Avenue, Chevy Chase, MD 20815 (US). GOLD, Philip, W. [US/US]; 3704 Quebec Street, Washington, DC 20016 (US). PAGE, Samuel, W. [US/US]; 500 Beaumont Road, Silver Spring, MD 20904 (US).		(74) Agents: MURPHY, Gerald, M., Jr. et al.; Birch, Stewart, Kolasch & Birch, P.O. Box 747, Falls Church, VA 22046 (US). (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: EVALUATIVE MEANS FOR DETECTING INFLAMMATORY REACTIVITY (57) Abstract <p>The present invention is directed to a method for testing the susceptibility of a mammal to inflammatory diseases which comprises the steps of: administering to a mammal a compound selected from the group consisting of Type I mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists or mixed estrogen agonists/antagonists, progesterone agonists; or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist which is effective in stimulating the hypothalamic-pituitary-adrenal (HPA) axis; and measuring the level of at least one hormone secreted by the hypothalamus, pituitary or adrenal glands of said mammal. The present invention is also directed to methods of treating inflammatory diseases and atypical depression.</p>		

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EVALUATIVE MEANS FOR DETECTING INFLAMMATORY REACTIVITY

BACKGROUND OF THE INVENTION

The present invention relates to a diagnostic test and kits for testing the susceptibility of individuals to inflammatory diseases such as rheumatoid arthritis with therapeutic agents directed at the central nervous system (CNS), designed to by-pass the CNS defect. The present invention is also directed to a method of treating inflammatory disease and atypical depression.

SUMMARY OF THE INVENTION

The invention is useful as a model in the study of the mammalian autoimmune diseases. Laboratory animals which may serve as a good model in studying human systems include rats, mice, guinea pigs, rabbits and chickens. However, an objective of this invention is to provide a method for diagnosing the susceptibility of humans to inflammatory diseases.

The hormones to be measured should be hormones which are secreted in increased levels by normal individuals when the compound is administered to the individual but which are not secreted in such high levels after administration of the compound in individuals having an inflammatory disease or susceptibility to an inflammatory disease. Hormones secreted by the pituitary and adrenal glands which

can be measured include glucocorticoids such as corticosterone, cortisol, and ACTH. Other hormones which
5 can be measured include CRH, prolactin, arginine vasopressin (AVP), growth hormone (GH), thyroid stimulating hormone (TSH), and endorphins/enkephalins. The substance which is administered should not be the same as the material which is measured.

10 The compound which is used in the test is preferably administered intravenously (i.v.), however, other modes of administration such as subcutaneously (s.c.) or orally (p.o.) may be used. The compound is administered together with a suitable non-toxic pharmaceutically acceptable
15 carrier in an amount sufficient to stimulate the hypothalamic-pituitary-adrenal axis. The compound should be administered at a time when the hypothalamic-pituitary-adrenal axis is quiescent, i.e., in humans at 8 p.m.; although it could be administered between 8 a.m.- 10 a.m.,
20 for example, when giving compounds such as AVP. When an immune/inflammatory mediator such as interleukin-1 is used, the immune inflammatory mediator would probably be administered in a dose of 0.1 $\mu\text{g/kg}$ to 10 $\mu\text{g/kg}$ of body weight, preferably 1 $\mu\text{g/kg}$ to 5 $\mu\text{g/kg}$ of body weight. When
25 CRH is used, 1 μg ovine CRH per kg body weight is administered i.v. When AVP is used, 0.01 to 2.0 mIU/kg/min is infused i.v. When a biogenic amine or analogue thereof such as quipazine is used, the compound would probably be administered in a dose of 0.01 to 1 mg of quipazine per kg
30 of body weight, preferably 0.1 to 0.5 mg/kg of body weight. Doses for other biogenic amines or analogues thereof should be determined on a case-by-case basis. When a narcotic antagonist such as naloxone is used, the compound would probably be administered in a dose of between about 25
35 mcg/kg to 175 mcg/kg, i.v.

After administration of the compound it is necessary to wait for a time sufficient to allow the compound to raise the glucocorticoid or ACTH level in the blood plasma of the patient before testing. Generally, it is necessary

to wait at least 10 minutes before testing. The glucocorticoid or ACTH level should be measured before the level returns to normal. The glucocorticoid or ACTH level may return to normal within 4 hours after administration of the compound. A preferred waiting period is 15 minutes to 2 hours after administration, more preferably 30 to 60 minutes after administration. If the hormone levels are significantly lower than (such as more than two standard deviations below) the mean established in normal individuals, then the patient has tested positive for possible susceptibility to inflammatory diseases.

The method is potentially useful for testing for inflammatory diseases including, but not limited to, arthritis, uveoretinitis, pneumonitis, encephalomyelitis, myocarditis, thyroiditis, nephritis, sialadenitis, adrenalitis, orchitis, multiple sclerosis and hepatic granulomatous diseases.

The present invention is also directed to a method of treating atypical depression, which comprises administering to a patient suffering from atypical depression, a compound which stimulates the hypothalamic-pituitary-adrenal axis in an amount effective to stimulate said axis.

The present invention is also potentially useful as a guide for the treatment of arthritis with agents that may bypass the HPA defect by stimulating the HPA axis centrally or at multiple levels, such as at the synovial level, i.e., synovial receptor binding. Such drugs would include the drugs listed below:

Neurotransmitters/monoamines/neuroexcitatory agents:

serotonin agonists/releasers/uptake inhibitors:

quipazine

1-metachloro-phenyl-piperazine (mCPP)

fenfluramine

fluoxetine

adrenergic agonists/antagonists/uptake inhibitors:

idasoxan

yohimbine

methoxamine

desmethylinipramine

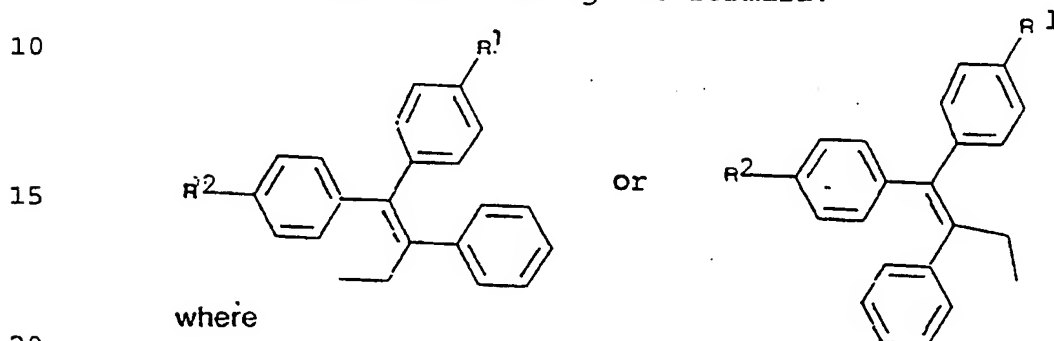
ritalin

- cholinergic agents:
 arecholine
 nicotine
- 5 GABA agonists/antagonists:
 BCCM-beta carboline
 FG 7142 (Sandoz)
 RO 15788
- 10 MAO inhibitors:
 MAO A:chlorgyline
 MAO B: phenylzine
 isocarboxazid
 tranylcypromine
- 15 Dopamine uptake inhibitors/releasers:
 bupropion
 amphetamine
- 20 Excitatory amino acids/neuroexcitatory agents;
 glutamate
 cocaine
- 25 Neurohormones:
 rat/human corticotropin releasing hormone (CRH)
 corticotropin (ACTH)
 dexamethasone
 arginine vasopressin (AVP)
 thyroxine
30 thyroid stimulating hormone (TSH)
 estrogen
 progesterone
 testosterone
 Diapid (Bissendorff, LHRH antagonist)
- 35 Type 1 Mineralocorticoid Receptor Antagonists(63, 64):
- 40 mespirenone (Δ^1 -15 β , 16 β -methylene-
 spironolactone, ZK 94 679)
 7 α -methylthio analogue of mespirenone (ZK 97 894,
 Δ^1 -15 β , 16 β -methylene-7 α -methylthio analogue of
 spironolactone)
 15 β , 16 β -methylene-mexrenone (ZK 91 587)
 1 α , 2 α : 15 α , 16 α -dimethylene-spironolactone
 15 α , 16 α -methylene-spironolactone
45 1 α , 2 α : 15 β , 16 β -dimethylene-spironolactone
 Δ^1 -15 α , 16 α -methylene-spironolactone
 spironolactone
 7 α -alkyl spironolactones
 7 α -alkyl steroidal 17-spirosultines
50 RU 26 107 and derivatives of RU 26 107
 7 α -propyl spironolactones such as
 RU 26752 and its corresponding K salt- RU 28 318
 RU 29705 and corresponding K salt RU 29706

Opiate Antagonists:

naloxone
naltrexone

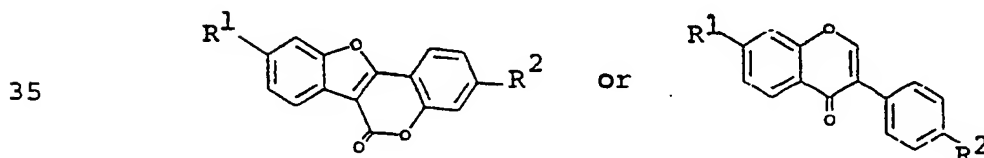
5 Estrogen antagonists, mixed estrogen agonists/
antagonists, progesterone agonists:
tamoxifen and related triphenylethylene derivatives
of tamoxifen having the formula:



20 $R^1 = \text{OH}, \text{OAc}, \text{OCH}_3, \text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2, \text{ or } \text{OCH}_2\text{CH}_2\text{NCS}$
 $R^2 = \text{H}, \text{OH}, \text{OCH}_3, \text{ or } \text{OAc}$

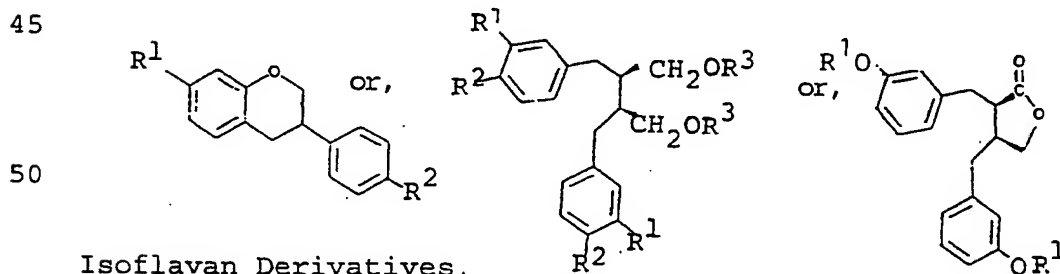
25 as described in D.W. Robertson and J.A. Katzenellebogen, J. Org. Chem., 47, 2387-2393 (1982); M. Schneider, J. Med. Chem., 29, 1494-1498 (1986).

30 phytoestrogens, such as coumestrol and related compounds having the formulae:



40 6-H-Benzofuro[3,2-c]-[1]-benzopyran-6-one
Derivatives, where
 $R^1 = \text{OH}, \text{OAc}, \text{ or } \text{OMe}$
 $R^2 = \text{OH}, \text{OAc}, \text{ or } \text{OMe}$

Isoflavone Derivatives,
where
 $R^1 = \text{OH}, \text{OAc}, \text{OGlu}, \text{ or } \text{OMe}$
 $R^2 = \text{OH}, \text{OAc}, \text{ or } \text{OMe}$



Isoflavan Derivatives,
where
55 $R^1 = \text{OH}, \text{OAc}, \text{ or } \text{OMe}$

where
 $R^1 = \text{OH}, \text{OMe}, \text{ or } \text{OAc}$

where
 $R^1 = \text{H}, \text{Ac},$

as described in U.S. Patent 2,890,116 and Kappe, Brandner, Z. Naturforsch., 29B, 292 (1974), and Walz, Ann., 489, 118 (1931).

progesterone agonists

5	norethindrone acetate	hydroxyprogesterone caproate
	norgestrel	medroxyprogesterone acetate
	levonorgestrel	norethynodrel
	norethindrone	megestrol acetate
	ethynodiol diacetate	

10

Combination therapy:

Since the mixed Type II glucocorticoid receptor/progesterone receptor antagonist RU 486 is potent in inducing inflammatory disease susceptibility to streptococcal cell walls in the F344/N rat, the present invention also includes a treatment regimen for rheumatoid arthritis which includes a combination of an estrogen antagonist with one or more of the following:

20

a Type I glucocorticoid receptor antagonist, such as mespironone
a Type II glucocorticoid agonist, such as prednisone and hydrocortisone, and
a progesterone agonist

25

It may also provide a guide for determination of dosage and timing schedule of replacement steroids or other HPA axis hormones such as CRH or ACTH.

The above noted agents have been selected for possible treatment of inflammatory diseases such as rheumatoid arthritis because they represent a variety of classes of neuroactive agents which would be expected to activate the CRH and/or related arousal systems on a long-term basis (i.e., without inducing tolerance). Such an effect would correct the putative pathophysiological defect in rheumatoid arthritis and, hence, significantly ameliorate inflammatory and/or affective symptoms associated with this illness. Of the above-mentioned compounds, it is expected that 1-metachloro-phenyl-piperazine (mCPP) (an antidepressant), fluoxetine (an antidepressant), idasoxan (an antidepressant), nicotine, FG 7142 (Sandoz), MAO A:chlorgyline (an antidepressant), and MAO B:phenylzine (an antidepressant) are expected to be preferred compounds for

further study. These compounds will be administered in the same manner and dosage range as recommended for their already known indications. Specifically, these compounds should be administered in an amount effective to stimulate the HPA axis which would bypass or overcome the defect in the HPA axis. For example, tamoxifen can be administered in the dosage amount of up to 20 mg/day total in humans. For example, naltrexone can be administered in the dosage amount of up to 50 mg/day in humans. It is also expected that analogues and/or derivatives of the above compounds may be useful.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A. Influence of mespirenone on carrageenan pouch concentration of white blood cells (WBC counts per ml). Lewis rats were treated subcutaneously for 8 weeks with either mespirenone, administered in emulphor vehicle, at 3 mg/kg, or vehicle control (emulphor). At the end of the treatment period, both groups of rats were challenged with carrageenan subcutaneously in an air pouch (67). Briefly, rats were slightly anaesthetized with CO₂. The skin of the dorsal surface of neck was shaved and cleaned with alcohol, and twelve milliliters (mL) of sterile air was subcutaneously injected to produce a well defined cavity. 24 hours later, 4 mL of 2% carrageenan (Sigma) was injected into the cavity that has already been formed (2% carrageenan, made up in sterile 0.9% NaCl). Rats were killed 24 hours later, and the carrageenan induced inflammatory exudate was aspirated from the pouch through a small incision. The volumes, white blood cell counts and differential were then analyzed. Data are presented as WBC (10³/ml) ± standard error of the mean (S.E.). Solid bar represents rats treated chronically with emulphor; hatched bar represents rats treated with mespirenone.

Figure 1B. Influence of mespirenone on carrageenan pouch total white blood cell counts. Rats were treated as described above, but two additional groups were included,

one chronically treated with mespirenone, and the other chronically treated with emulphor. At the termination of the study, these two additional groups were challenged with saline in the air pouches rather than carrageenan (L/SAL (+) = mespirenone treated group; L/SAL (-) = emulphor treated group). Rats chronically treated with mespirenone, and challenged with carrageenan = L/CAR (+); rats chronically treated with emulphor and challenged with carrageenan = L/CAR (-). Results are expressed as mean total white blood cell count in exudates $\times 10^4$.

Figure 2. Influence of mespirenone on CRH content in LEW/N rats after carrageenan challenge. Rats were treated as described in Figures 1A and 1B, and CRH content was measured in the median eminence. L/SAL (+) = mespirenone treated group, challenged with saline in air pouches; L/SAL (-) = emulphor treated group, challenged with saline in air pouches; rats chronically treated with mespirenone, and challenged with carrageenan = L/CAR (+); rats chronically treated with emulphor and challenged with carrageenan = L/CAR (-). Data are expressed as mean ng CRH.

Figure 3A. Effect of Tamoxifen on total leukocyte count in carrageenan pouch. Lewis rats were treated subcutaneously for 3 weeks with either Tamoxifen, administered in emulphor vehicle, at 1 mg/Kg, or vehicle control (emulphor). At the end of the treatment period, both groups of rats were challenged with carrageenan subcutaneously in an air pouch (5), as described above (Figures 1A and 1B). Results are expressed as the mean of the total leukocytosis (total white blood cell count) mean \pm standard error of the mean (S.E.M.).

Figure 3B. Effect of Tamoxifen on leukocyte concentrations (WBC/ml) in carrageenan pouch. Lewis rats were treated subcutaneously for 3 weeks with either Tamoxifen, administered in emulphor vehicle, at 1 mg/Kg, or vehicle control (emulphor). At the end of the treatment period, both groups of rats were challenged with carrageenan subcutaneously in an air pouch (5), as

described above (Figures 1A and 1B). Results are expressed as the mean of the white blood cell count (WBC) $\times 10^6/\text{ml} \pm$ standard error of the mean (S.E.M.). Statistical significance was determined using the Scheffe's super ANOVA. *=significant ($p < 0.0015$).

Figure 3C. Effect of Tamoxifen on volume of exudate (ml) in carrageenan pouch. Lewis rats were treated subcutaneously for 3 weeks with either Tamoxifen, administered in emulphor vehicle, at 1 mg/Kg, or vehicle control (emulphor). At the end of the treatment period, both groups of rats were challenged with carrageenan subcutaneously in an air pouch (5), as described above (Figures 1A and 1B). Results are expressed as the mean volume (ml) \pm standard error of the mean (S.E.M.). Statistical significance was determined using the Scheffe's super ANOVA. *=significant ($p < 0.03$).

Figure 4A. Peak plasma ACTH responses in normal control human volunteers following infusion of naxolone at various doses i.v. Samples were collected at 15 min sampling intervals for 2 hours after the infusion. Peak response fell between 45 and 90 minutes after start of infusion.

Figure 4B. Percent above baseline of peak plasma ACTH responses in normal control human volunteers following infusion of naxolone at various doses i.v. Samples were collected at 15 min sampling intervals for 2 hours after the infusion. Peak response fell between 45 and 90 minutes after start of infusion.

Figure 5. Effect of tamoxifen 0.1 mg/Kg, 1 mg/Kg, or 10 mg/Kg on total leucocyte count in carrageenan pouches. Lewis rates were treated subcutaneously for 3 weeks with either Tamoxifen, administered in emulphor vehicle, at 1 mg/Kg, or vehicle control (emulphor). AT the end of the treatment period, both groups of rats were challenged with carrageenan subcutaneously in an air pouch (5), as described above (Fig. 1A and 1B). Results are expressed as the mean volume (ml) \pm standard error of the mean (S.E.M.).

Stastical significance was determined using the Scheffe's super ANOVA.* = significant ($p < 0.03$). Rats treated with tamoxifen 0.1 mg/Kg or 1 mg/Kg showed statistically significant decrease in inflammatory response as
5 quantitated by total leucocyte count in pouches ($p < 0.001$).

DETAILED DESCRIPTION OF THE INVENTION

Arginine Vasopressin Stimulation Test:

There are currently two methods available to test the
10 integrity of HPA axis responses in humans: the CRH stimulation test, and the arginine vasopressin (AVP) stimulation test (Meller, W.H. et al., J. of Psychiatric Research, 21, No. 3, 269-177 (1987)). Further details of this test can be found in NIH Clinical Project No. 87-M-85a
15 which is hereby incorporated herein be reference. Both of these methods have been developed at the NIH Clinical Center, and have been extensively studied in diseases such as depression and Cushings' syndrome. The CRH stimulation test determines sensitivity of ACTH and cortisol responses
20 to exogenous CHR, and therefore tests the integrity of pituitary corticotroph cells' reponses to stress. Since defective pituitary responsiveness to stress could be primary, or could be secondary to long-term hypothalamic defects as well, the CRH test also indirectly tests the
25 integrity of hypothalamic responses to stress. A more direct measure of the integrity of hypothalamic CRH responses to stress is the AVP stimulation test. AVP synergizes with CRH in stimulating ACTH release. Decreased endogenous CRH would therefore result in decreased ACTH and
30 cortisol secretion in response to exogenous AVP. Maximal ACTH and cortisol responses to AVP stimulation occur at 9 a.m., when CRH should be at its peak. Maximal ACTH and cortisol responses to CRH stimulation occur when CRH is at its nadir, at 4 p.m. An intravenous infusion of arginine
35 vasoporessin of 1.0 mIU/kg/min administered over a one hour period between 9:00 and 10:00 a.m. has been shown to have minimal side effects, and to maximally synergize with

endogenous CRH in stimulating ACTH release. However, the infusion could be given at 8 p.m. to 9 p.m., depending on results of preliminary testing.

5 AVP Stimulating Test:

Arginine vasopressin will be administered by an intravenous infusion of 1.0 mIU/kg/min of arginine vasopressin over a one hour period between 9:00 and 10:00 a.m. The infusions will be by means of a digitally
10 controlled Extracorporeal Constant Infusion Pump, Model 2100. If any given dosage side effects such as nausea or gastrointestinal cramping occur, the infusion will be stopped immediately. Upon cessation of side effects, the infusion may again commence at a dose established to be
15 free of side effects for a given individual. Side effects should be rare even at the maximal doses proposed for this study. In addition to the standard intracath placed for infusion of arginine vasopressin, blood will be drawn through a scalp vein needle in the contralateral arm, every
20 15 minutes before, during, and for two hours after the infusion. Eight cc's will be taken for every sample, and a total of 104 cc's will be taken for each infusion. Blood will be assayed for ACTH and cortisol, and in some cases also for other hormones such as beta-endorphin, growth
25 hormone, prolactin and oxytocin.

Synthetic arginine vasopressin will be obtained from the Parke-Davis Company. Parke-Davis markets a highly purified synthetic peptide with a sequence of naturally occurring arginine vasopressin and the commercial
30 designation Aqueous Pitressin.

Treatment with Mespirenone:

In preliminary testing in Lewis (LEW/N) rats, the inventors have found that chronic treatment of these rats
35 with the mineralocorticoid (Type I glucocorticoid receptor) antagonist, mespirenone, (INN prop. for 7 α -Acetylthio-15 β , 16 β -methylen-3-oxo-17 α -pregna-1,4-dien-21,17-carbolactone)

significantly suppressed their inflammatory response to carrageenan, as determined by total leukocytes and leukocytes/cc present in subcutaneous carrageenan pouches (see Figures 1A and 1B). The drug was administered in
5 emulphor vehicle subcutaneously, at 3 mg/Kg, for 8 weeks. The inflammatory response to carrageenan in mespirenone treated rats was 50% less than in vehicle-treated control LEW/N rats ($p < 0.05$). At the same time, hypothalamic CRH mRNA tended to increase in mespirenone-treated rats
10 compared to saline-treated controls (Figure 2, consistent with the hypothesis that normalization of CRH responses is associated with suppression of inflammatory responses. The lack of increase in CRH mRNA in mespirenone-treated, carrageenan exposed animals, compared to non-mespirenone-
15 treated, carrageenan exposed animals, probably reflected the lower inflammation, in the drug-treated animals. Also consistent with the hypothesis that modification of CRH responses alters inflammatory responses, is the finding that relatively inflammatory disease resistant Fischer
20 (F344/N) rats exhibited significantly lower volumes of carrageenan exudates than LEW/N rats, consistent with the previous findings that they also exhibit significantly less inflammatory responses to streptococcal cell walls and other arthritogenic stimuli. Nonetheless, the small F344/N
25 inflammatory response to carrageenan was also inhibited by mespirenone by 50%, although due to small numbers of animals and small exudates, this value did not reach significance.

These data indicate that pharmacologic interruption of
30 the major inhibitory pathway of CRH regulation, glucocorticoid negative control via an antagonist to the Type 1 mineralocorticoid receptor, is associated with suppression of the inflammatory response in these otherwise highly inflammatory disease susceptible rats.

Treatment with Tamoxifen:

A previously healthy 69 year old female was diagnosed to have full blown acute onset rheumatoid arthritis, after a two month course of fever, night sweats (sometimes accompanied by chills), transient petechial maculopapular rashes, migrating polyarthralgias and arthritis in the small and large joints, including proximal interphalangeal joints (PIPs), metacarpal and metatarsalphalangeal joints (MCPs and MTPs), knees, wrists, elbows, shoulders and neck. One month prior to the onset of fever, the patient's initial symptoms had consisted of bilateral carpal tunnel syndrome, diagnosed on history and clinical exam, and confirmed by nerve conduction studies. Also, the patient had had a similar episode of fevers and sweats 3 1/2 years before, accompanied by a non-malignant axillary lymph node, described as reactive on excisional biopsy. No cause was ever identified for the episode, and it resolved spontaneously with no pharmacologic intervention post-excisional biopsy. Laboratory studies during the current episode revealed a newly positive serum rheumatoid factor (R.F.), elevated sedimentation rate (50 mm/Hr) and negative ANA and anti-DNA. Serum protein electrophoresis and C3 were normal. Chest x ray showed mild hilar adenopathy and mild pleural effusions, with no parenchymal disease and no evidence of lymphangitic infiltration on C.T. scan. Anergy screen was normal, ppd was negative, and angiotensin converting enzyme was normal. A tentative diagnosis of adult Still's disease was made, but allergic or serum sickness reaction, to non-steriodal anti-inflammatory agents used to treat the initial carpal tunnel syndrome, could not be ruled out.

In the course of the investigation of the fever of unknown origin and hilar adenopathy, the patient was found to have a mass in the left breast, with characteristic features of malignancy on physical examination and by mammography. On excisional biopsy, two primary ductal carcinomas, 2.1 cm and under 2.0 cm, were found, with

margins clear of tumor. Both tumors were strongly positive for estrogen and progesterone receptors. Metastatic work-up was negative, with negative bone scan, normal abdominal ultrasound, normal C.T. scan of the head and abdomen. The
5 breast cancer was classed as stage 2, on the basis of size of tumors and lack of metastatic spread. In light of the age of the patient and the severe and worsening systemic symptoms of the rheumatoid arthritis, further staging surgery and chemotherapy were deferred, and the patient
10 elected to be treated with a regimen of tamoxifen (20 mg/day) and local radiation to the breast.

Prior to local excisional surgery, the arthritis symptoms continued to worsen, with increasing severity and duration of pain and swelling in the involved joints, and
15 increasing numbers of joints simultaneously involved. In addition, although the fevers evolved from high spiking fevers to low grade more chronic fever, the systemic symptoms worsened, with day long stiffness, weakness, malaise, to the point that the week prior to surgery the
20 patient was bedridden most of the day and unable to turn in bed, get out of bed, or walk, without assistance. All medication was withheld prior to surgery, except for acetaminophen prn, because allergy to non-steroidal anti-inflammatory medication could not be ruled out as a
25 possible cause of the spiking fevers. Physical examination immediately post-operatively revealed synovitis of both wrists, 5th left PIP, right 1st MTP, and a marked (3+) effusion of the left knee. Both shoulders were painful on movement. Aspiration of the left knee revealed 25 mls of
30 cloudy fluid, positive for rheumatoid factor and negative on routine culture. At that time, a definite diagnosis of rheumatoid arthritis was made, and prednisone 20 mg QD was begun 5 days later.

The symptoms partially improved, but remained marked
35 after two weeks of prednisone 20 mg/day x 4 days, tapering to 15 mg/day for the remainder of time. At that time, tamoxifen was begun, for treatment of the breast cancer.

After 2 days of tamoxifen, and continued tapering of prednisone to 10 mg/day, the patient noted a dramatic improvement in symptoms, with decreased pain and swelling, and increased range of motion (ROM) in the shoulders, wrists, PIPs and MCPs and neck, but persistent effusion in the left knee. The systemic symptoms also improved, with general increased mobility, decreased duration of stiffness to less than 30 minutes, and resolution of fever. The symptoms continued to improve and physical examination after two weeks of tamoxifen and 10 mg/day prednisone treatment revealed full ROM in the left shoulder, 90% ROM in the right shoulder, slight swelling of the right wrist, minor swelling of the left 5th PIP, but persistent warmth, swelling, tenderness and effusion of the left knee. At that time, 1 cc methylprednisolone was instilled into the left knee, and tamoxifen 20 mg/day and prednisone 10 mg/day were continued. Radiation therapy to the left breast was begun 6 days later, and a full 5 week course carried out. Two months after initiating treatment, the patients remains largely free of joint symptoms on the current therapy, with occasional fleeting arthralgias in the shoulder and knee, 0 to less than 20 minutes morning stiffness, no fevers or rashes, and feels generally well, subjectively 80% improved overall.

25

Animal Studies:

In preliminary testing in Lewis (LEW/N) rats, the inventors have found that chronic treatment of these rats with the estrogen receptor antagonist, tamoxifen, significantly suppressed their inflammatory response to carrageenan, as determined by leukocytes/cc, total leukocytes and total volume of exudate present in subcutaneous carrageenan pouches (see Figures 3A-3C attached). The drug was administered in emulphor vehicle subcutaneously, at 1 mg/Kg, for 3 weeks. The inflammatory response to carrageenan in tamoxifen treated was 75%-80% less than in vehicle-treated control LEW/N rats ($p < 0.002$).

These data indicate that the pharmacological treatment with the estrogen antagonist tamoxifen alone or in combination with the Type II glucocorticoid agonist prednisone significantly suppresses inflammatory responses and/or arthritis.

Synovial Receptor Binding:

In order to determine which combination of agents would be most effective in a given patient, synovial tissue should be biopsied, and estrogen, progesterone, glucocorticoid Type 1 and glucocorticoid Type 2 receptor binding number and affinity will be determined by standard receptor binding techniques (65, 66).

Kits:

The components for use by the means described herein can be assembled in kits for use in accord with the teachings of the application. Such kits may contain the hypothalamic-pituitary-adrenal (HPA) axis stimulating drug, tracers such as I^{125} or an ELISA tracer, reagents specific for use in the assay chosen, antibodies to HPA axis hormone, and, for use as controls, standardized plasma. Any of the HPA axis hormones such as GABA agonists/antagonists, MAO inhibitors, Dopamine uptake inhibitors/releasers, Cholinergic agents, serotonin agonists/releasers/uptake inhibitors, adrenergic agonists/antagonists/uptake inhibitors, neurohormones, Type 1 mineralocorticoid receptor antagonists, estrogen antagonists, progesterone agonists, named at pages 5-6 are appropriate for use in kits in accord with the teachings of this disclosure. 1-metachloro-phenyl-piperazine (mCPP) is a particularly preferred stimulant.

Diseases of the Stress Response II: Atypical Depression:

Overview: In contrast to the intense hyperarousal characteristic of melancholic depression, the syndrome of atypical depression seems to represent an excessive

counter-regulation of the generalized stress response (68). The facilitation of pathways subserving arousal and attention in melancholic depression is replaced with apathy, lethargy, and passivity in atypical depression.

5 Similarly, the inhibition of pathways subserving vegetative functions in melancholia contrasts with the hyperphagia and hypersomnia that are among the defining characteristics of atypical depression. This syndrome is not only frequently diagnosed as a primary psychiatric (depressive) illness,
10 but also commonly occurs across the boundaries of a variety of medical illnesses including Cushing's disease, (75) the Chronic Fatigue Syndrome (78), hypothyroidism (77), and seasonal affective disorder (76).

15 Examples of atypical depression include:

Cushing's Disease:

Several lines of data in preoperative and post-operative Cushing's disease patients indicate that the CRH neuron has been suppressed by longstanding
20 hypercortisolism that derives from a peripheral (pituitary) abnormality. These data include responses to CRH (71, 79), the effects of priming on the corticotroph cell In post-operative patients (79), and measurements of CSF CRH (75).

25

Chronic Fatigue Syndrome:

The chronic fatigue syndrome is defined by the Center for Disease Control (CDC) as an illness consisting of profound debilitating fatigue lasting six months or longer
30 in the absence of any clearly definable systemic illness, and often associated with feverishness, myalgias, arthralgias, and high titers to a variety of viral antigens (78). In a study of 30 patients meeting CDC criteria for the Chronic Fatigue Syndrome (CFS) followed longitudinally
35 for over one year at the NIH Clinical Center, it has been shown that the lethargy and fatigue in patients with the CFS occur in the context of a hypofunctioning CRH neuron

(78). Hence, it has been shown that despite a significant reduction in evening basal total and free cortisol levels and in 24-hour urinary free cortisol excretion, patients with the CFS showed blunted ACTH responses to ovine CRH.

5 It has also been shown that patients with the CFS showed exaggerated cortisol responses to low doses of ACTH and blunted cortisol responses to high-dose ACTH administration. These data suggest that in the context of a subtle central adrenal insufficiency, adrenocortical ACTH

10 receptors have grown hyperresponsive to ACTH, but that because of an atrophy of the adrenal cortex due to a central CRH deficiency, the adrenocortical response to high doses of ACTH is attenuated. This pattern of response has previously been seen in patients receiving alternate day

15 glucocorticoid treatment and known to have a partial central adrenal insufficiency on this account.

The symptomatology of the CFS could not only be facilitated by a CRH deficiency, but also by hypocortisolism per se. Hence, hypocortisolism is not only

20 classically associated with fatigue, but also with feverishness, myalgias, and arthralgias. Moreover, in the light of the data advanced regarding the role of endogenous HPA function on immune functions (80, 81), hypocortisolism could also be associated with a generalized increase in

25 immunologic function, including increased titers to a variety of viral antigens.

Seasonal Affective Disorder. Patients with seasonal affective disorder present with depressions characterized

30 by hyperphagia and hypersomnia. It has been demonstrated that a significant attenuation and delay in the ACTH response is due to ovine CRH. This delayed pattern is analogous, in part, to what was described in patients with central adrenal insufficiency secondary to trauma or tumor,

35 and, in part, to data in patients with Cushing's disease studied after transsphenoidal hypophysectomy with post-operative adrenal insufficiency (76).

Hypothyroidism:

Hypothyroidism, is often associated with symptoms of atypical depression. It has been demonstrated that patients with hypothyroidism show responses to ovine CRH compatible with central adrenal insufficiency that consist of exaggerated, delayed ACTH responses to CRH, in association with a decreased cortisol response to the ACTH released during the course of the CRH stimulation test.

10 An animal model for the effects of experimentally-induced hypothyroidism on the functional integrity of the HPA axis in the rat:

In hypothyroid rats, a variety of in vivo and in vitro techniques to demonstrate the presence of a central, CRH-mediated adrenal insufficiency have been utilized. In vitro studies show that hypothyroid rats show a decrease in PVN CRH RNA and content, and a decrease in KCl-induced CRH release from hypothalamic organ culture. A concomitant decrease in the number of hippocampal glucocorticoid receptors has been demonstrated. Studies in pituitary cell culture show a decrease in POMC RNA and ACTH content, an increase in CRH receptors, and an increased ACTH response of dispersed pituicytes to CRH. Adrenal weights were significantly decreased and there was a subnormal corticosterone response to ACTH by cultured adrenal cells. Corroborating in vivo studies in hypothyroid rats show a significant decrease in CSF and plasma free cortisol concentrations and several findings compatible with central adrenal insufficiency. These include an attenuated response to the central CRH stimulus IL-1, an exaggerated ACTH response to CRH in accordance with an increase in CRH receptors and decreased glucocorticoid negative feedback, and decreased responsiveness of the adrenal cortex to ACTH.

Animal models of melancholic and atypical depression
in histocompatible Lewis (LEW/N) and Fisher (F344/N) rats:

It has been previously shown that susceptibility to inflammatory in the LEW/N rat reflects hypoactivity of the hypothalamic CRH neuron to a variety of stimuli, including not only inflammatory mediators but environmental stimuli as well (80, 81). Hence, in response to inflammatory triggers, LEW/N rats fail to recruit the central nervous system CRH neuron to activate pituitary-adrenal function, and hence, glucocorticoid mediated counter-regulation of the immune response. It has also been demonstrated that histocompatible F344/N rats show resistance to inflammatory disease based on a hyperresponsiveness of their CRH neuron and pituitary- adrenal axis to inflammatory mediators. In the light of data that the central administration of CRH to experimental animals produces many changes that promote successful adaptation during threatening situations (e.g. not only pituitary-adrenal activation, but also activation of the sympathetic nervous system, enhancement of arousal, alertness, anxiety, inhibition of growth, feeding, and reproduction), the inventors also explored whether the relative hypo-and hyper-active of the CRH neurons in LEW/N and F344/N rats was associated not only with differences in susceptibility to inflammatory disease, but differences in behavior as well. Data shows that CRH deficient LEW/N rats behave like outbred rats given CRH antagonists. They fail to respond appropriately to stressful stimuli with reduced exploration, and anxiety and they are lethargic in the face of threatening situations. Conversely, F344/N rats respond like outbred rats given CRH, and show enhanced arousal, decreased exploration, and hyperactivity. In this regard, the CRH deficiency of the LEW/N rat is associated with a behavioral state analogous to the lethargy and fatigue seen in illnesses characterized by atypical depression. Conversely, the CRH hypersecretion in the F344/N rats is associated with behavioral arousal analogous to that seen in melancholic depression. Data shows that type I

glucocorticoid receptor antagonists such as mepirone are capable of significantly elevating CRH content in the hypothalamus (see Figure 2) suggests that drugs like this which were predicted to enhance CRH synthesis could be
5 useful in the treatment of atypical depression syndromes, whether occurring de novo or in association with illnesses such as chronic fatigue syndrome or rheumatoid arthritis. In this regard, inflammatory illnesses like rheumatoid arthritis are known to be associated with a
10 disproportionately high incidence of atypical depressive features.

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WHAT IS CLAIMED IS:

1. A method for testing the susceptibility of a mammal to inflammatory diseases which comprises the steps of:

administering to a mammal a compound selected from the group consisting of Type 1 mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists or mixed estrogen agonists/antagonists, progesterone agonists; or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist which is effective in stimulating the hypothalamic-pituitary-adrenal (HPA) axis; and

measuring the level of at least one hormone secreted by the hypothalamus, pituitary or adrenal glands of said mammal.

2. The method of claim 1, which comprises the steps of administering to a mammal a compound selected from the group consisting of Type 1 mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists or mixed estrogen agonists/antagonists, progesterone agonists; or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist, and measuring the level of glucocorticoids or

adrenocorticotrophic hormone or corticotrophic releasing hormone (CRH) in said mammal.

3. The method of claim 1, wherein said inflammatory disease is arthritis, uveoretinitis, pneumonitis, encephalomyelitis, multiple sclerosis or hepatic granulomas.

4. The method of claim 1, wherein said compound is mespirenone or tamoxifen; or a pharmaceutically acceptable salt thereof, and said hormone is cortisol, corticosterone, adrenocorticotrophic hormone or corticotrophic releasing hormone (CRH).

5. The method of claim 1, wherein the level of said hormones secreted by the hypothalamus, pituitary or adrenal glands are measured 10 minutes to 4 hours after administration of said compound.

6. A method for testing the susceptibility of a mammal to arthritis which comprises the steps of:

administering to a mammal an amount of mespirenone or tamoxifen effective to stimulate the hypothalamic-pituitary-adrenal axis; and

measuring the level of CRH adrenocorticotrophic hormone or corticosterone in the plasma of said mammal between 10 minutes and 4 hours after administration of said mespirenone or tamoxifen.

7. The method of claim 6, wherein said mammal is a laboratory animal.

8. The method of claim 6, wherein said mammal is a human.

9. A method for treatment of inflammatory diseases which comprises the step of:

administering to a patient suffering from an inflammatory disease, an effective amount of a compound which stimulates the hypothalamic-pituitary-adrenal axis which is selected from the group consisting of Type 1 mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists, or mixed estrogen agonists/antagonists, progesterone agonists; or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist.

10. The method of Claim 9, wherein said compound is administered to a patient suffering from rheumatoid arthritis.

11. A kit comprising:

(a) a HPA axis stimulating agent selected from the group consisting of Type 1 mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists, mixed estrogen agonists/antagonists, progesterone agonists;

or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist, and measuring the level of glucocorticoids, adrenocorticotrophic hormone or CRH; and

(b) reagents for measuring a HPA axis hormone response.

12. The kit of Claim 11 further comprising a tracer.

13. The kit of Claim 11, further comprising antibodies to the HPA axis hormone.

14. The kit of Claim 11, wherein said reagents for measuring the HPA axis hormone response comprise reagents for an ELISA test.

15. The kit of Claim 14 further comprising an ELISA tracer.

16. The kit of Claim 13, further comprising a standardized plasma as a control.

17. The kit of Claim 12, wherein said tracer is I^{125} .

18. The method of Claim 9, wherein said compound is mespirenone.

19. The method of Claim 2, wherein said compound is an opiate antagonist selected from the group consisting of naloxone and naltrexone.

20. The method of Claim 2, wherein said compound is an estrogen antagonist selected from the group consisting of tamoxifen and triphenylethylene derivatives of tamoxifen, phyto-estrogens and progesterone agonists.

21. The method of Claim 2, wherein said glucocorticoid is selected from the group consisting of cortisol and corticosterone.

22. The method of Claim 2, wherein said measuring of glucocorticoids, adrenocorticotrophic hormone or CRH is in blood plasma.

23. The method of Claim 2, wherein said inflammatory disease is arthritis.

24. The method of Claim 9, wherein said compound is an opiate antagonist selected from the group consisting of naloxone and naltrexone.

25. The method of Claim 9, wherein said compound is an estrogen antagonist or mixed estrogen agonist/antagonist is selected from the group consisting of tamoxifen and

triphenylethylene derivatives of tamoxifen, phyto-estrogens and progesterone agonists.

26. The kit of Claim 11, wherein said stimulating agent is an opiate antagonist selected from the group consisting of naloxone and naltrexone.

27. The kit of Claim 11, wherein said stimulating agent is an estrogen antagonist or mixed estrogen agonist/antagonist is selected from the group consisting of tamoxifen and triphenylethylene derivatives of tamoxifen, phyto-estrogens and progesterone agonists.

28. A method of treating atypical depression, which comprises administering to a patient suffering from atypical depression, a compound which stimulates the hypothalamic-pituitary-adrenal axis in an amount effective to stimulate said axis.

29. The method of Claim 28, wherein said compound is a mineralocorticoid receptor antagonist selected from the group consisting of Type 1 mineralocorticoid receptor antagonists, opiate antagonists, estrogen antagonists, mixed estrogen agonists/antagonists, progesterone agonists; or a combination of an estrogen antagonist with one or a combination of a Type I glucocorticoid receptor antagonist, a Type II glucocorticoid agonist or a progesterone agonist.

1/6

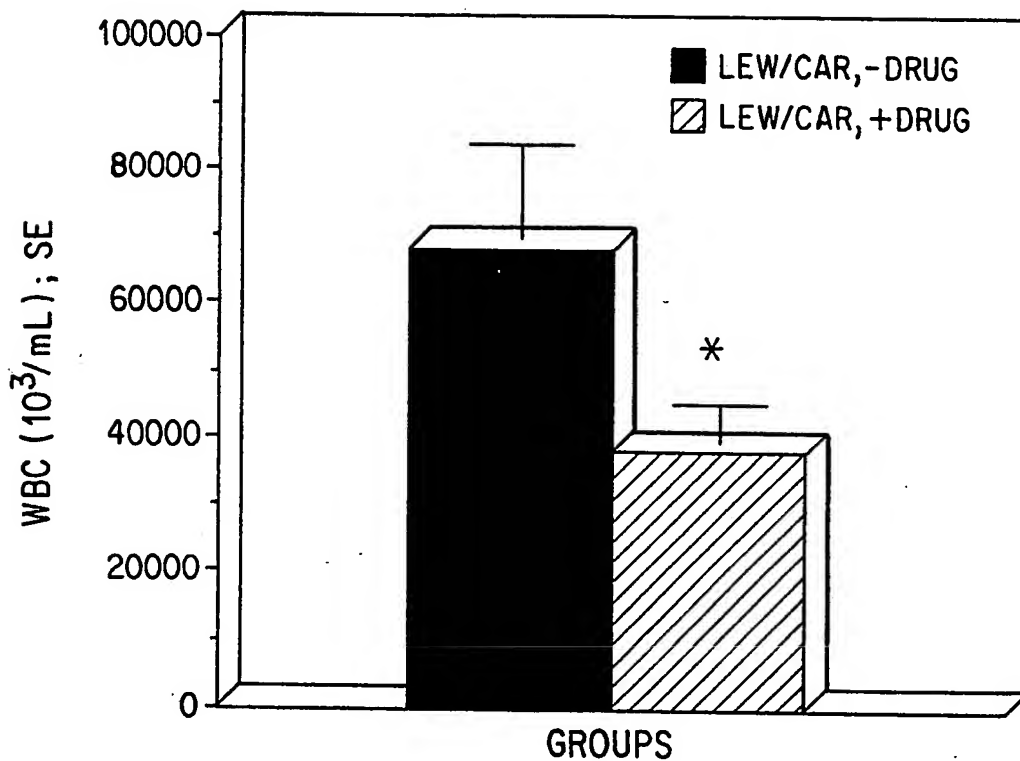


FIG. 1A

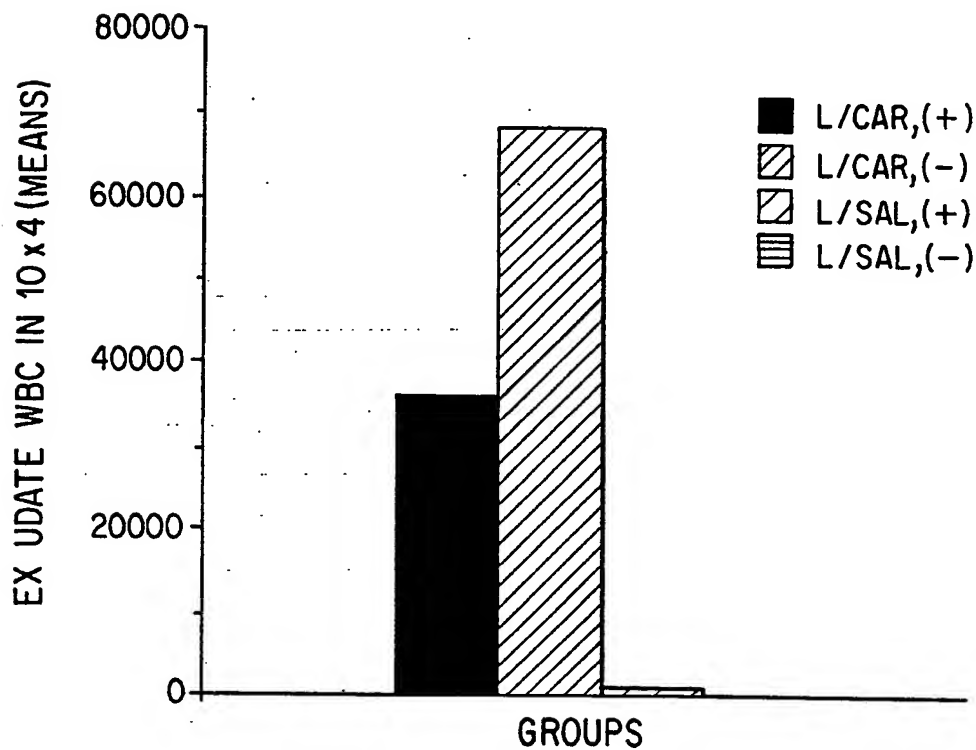


FIG. 1B

2/6

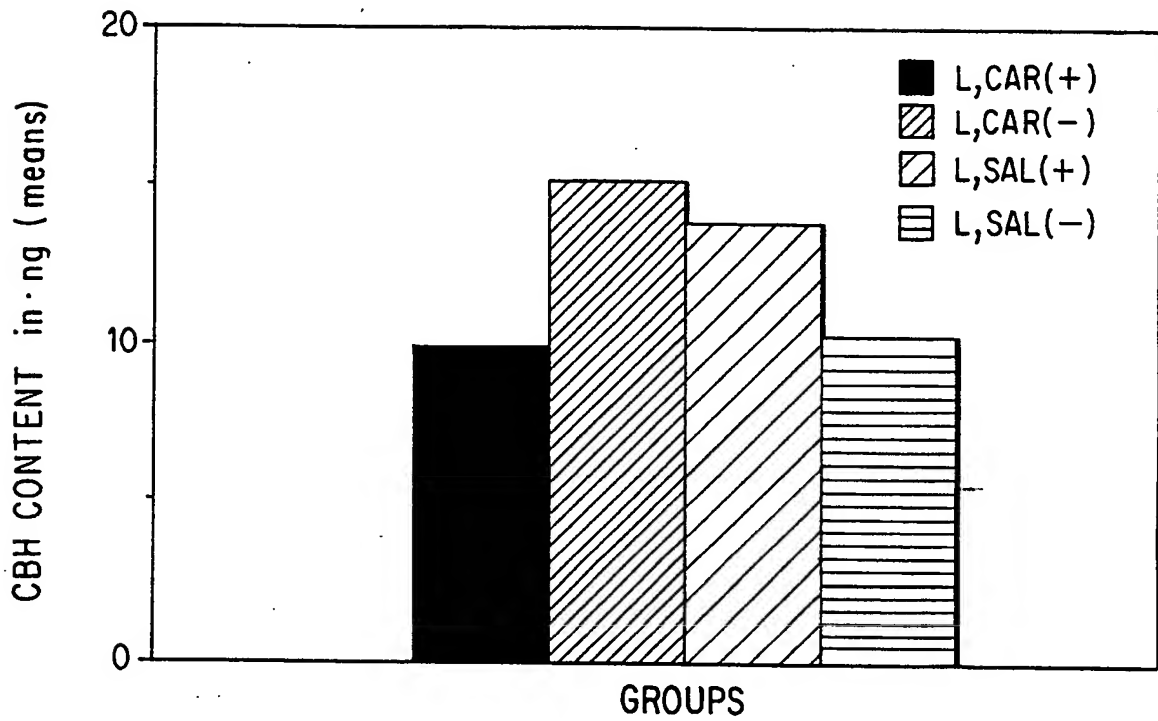


FIG. 2

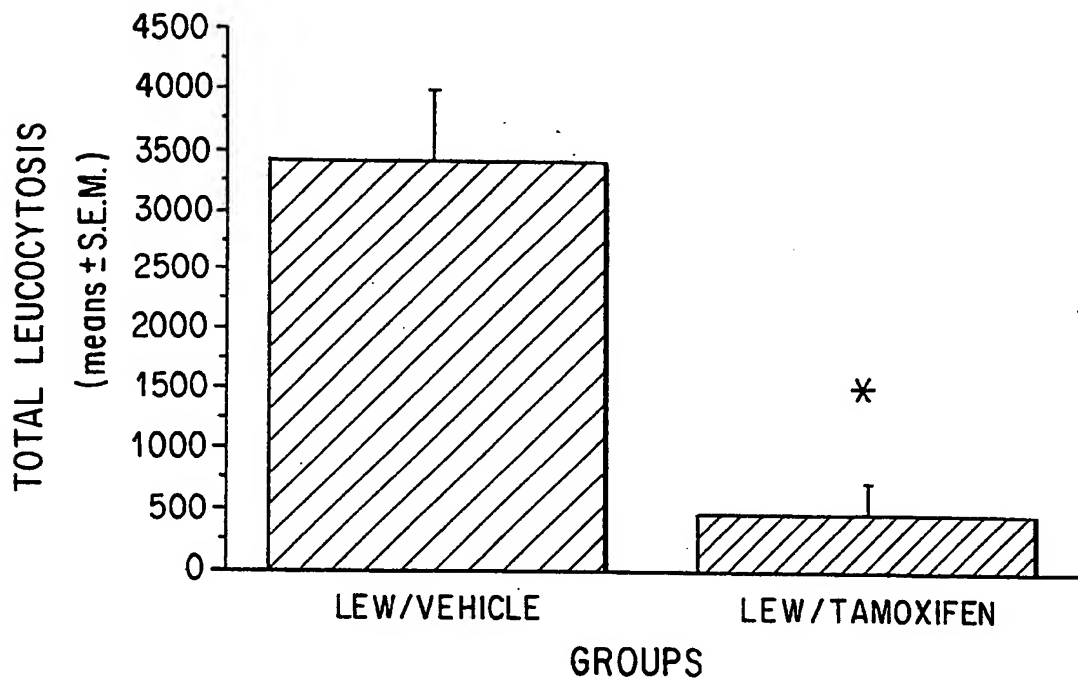


FIG. 3A

SUBSTITUTE SHEET

3/6

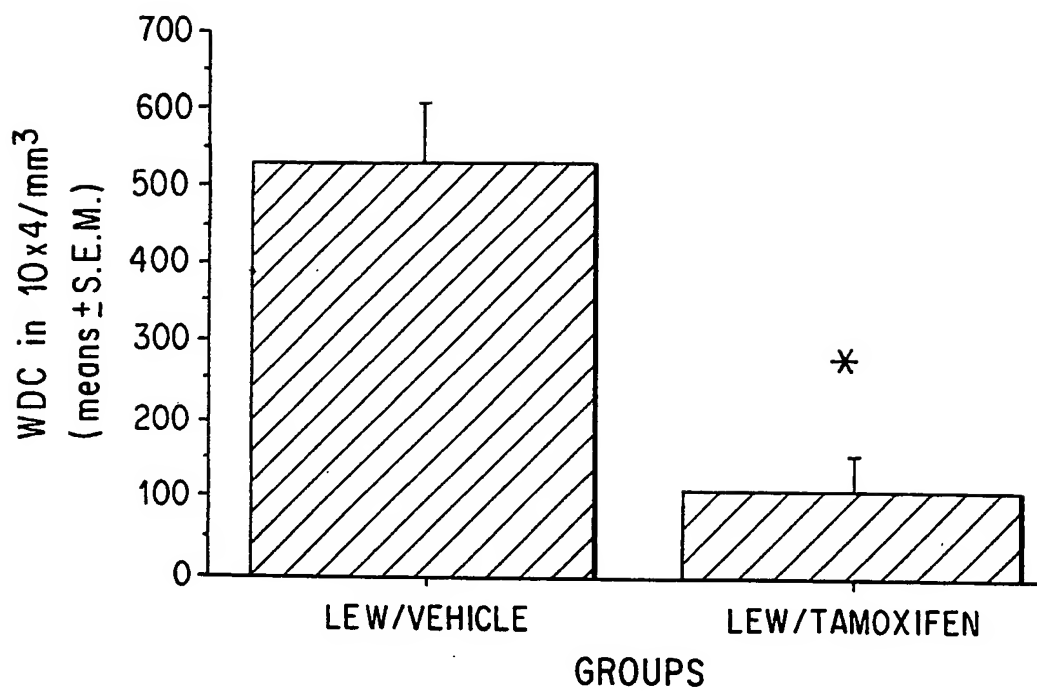


FIG. 3B

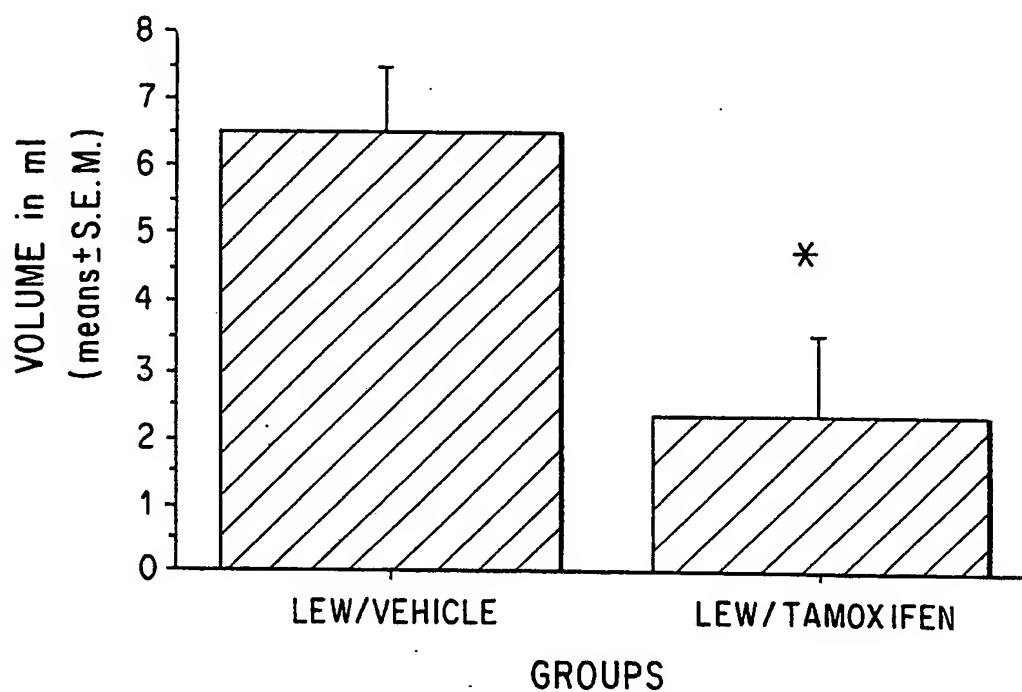


FIG. 3C

SUBSTITUTE SHEET

4/6

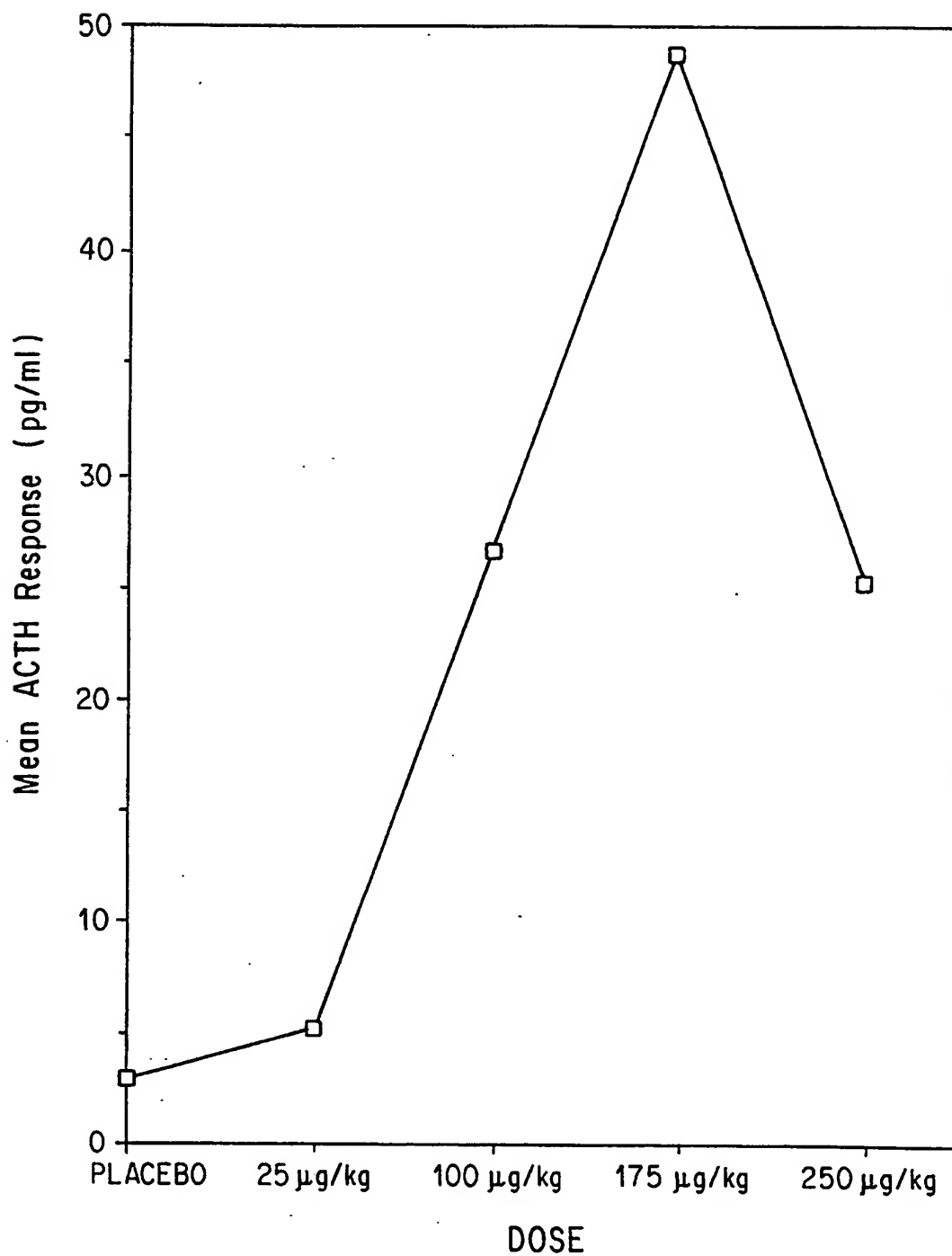


FIG. 4A

5/6

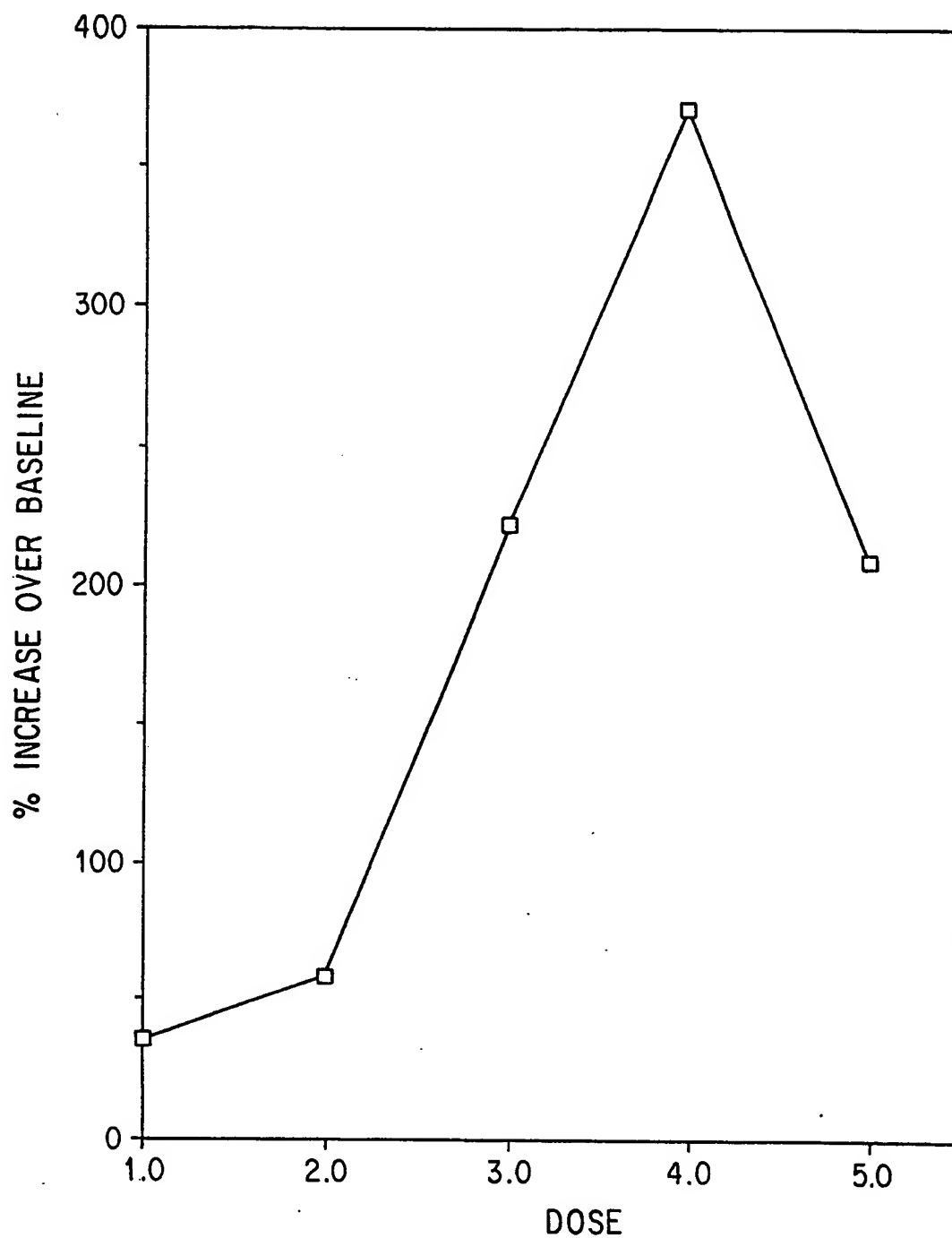


FIG. 4B

6/6

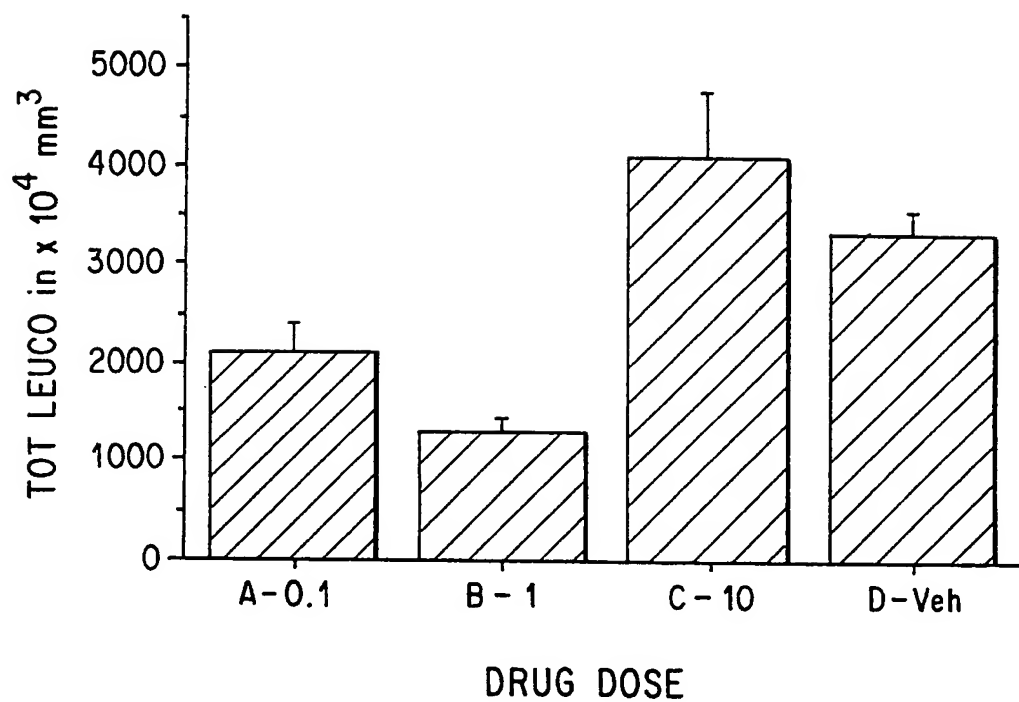


FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/04070

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 G01N33/74; A61K31/135;	G01N33/58; A61K31/565;	A61K45/06; A61K31/57 A61K31/485
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	G01N ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 86, no. 7, April 1989, WASHINGTON US pages 2374 - 2378 ESTHER M. STERNBERG ET AL. 'Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats' cited in the application	1-23
Y	see the whole document --- -/--	1-29
<p>¹⁰ Special categories of cited documents : ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
31 AUGUST 1993	14. 09. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	DÖPFER K.P.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 86, no. 12, June 1989, WASHINGTON US pages 4771 - 4775 ESTHER M. STERNBERG ET AL. 'A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats' cited in the application	1-23
Y	see the whole document	1-29
Y	THE NEW ENGLAND JOURNAL OF MEDICINE vol. 314, no. 21, 22 May 1986, BOSTON MA US pages 1329 - 1335 PHILIP M. GOLD ET AL. 'Responses to Corticotropin-Releasing Hormone in the Hypercortisolism of Depression and Cushing's Disease' cited in the application see the whole document	1-29
Y	CLINICAL ENDOCRINOLOGY & METABOLISM vol. 72, no. 2, February 1991, LONDON GB pages 260 - 271 MITCHEL A. KLING ET AL. 'Cerebrospinal Fluid Immunoreactive Corticotropin-Releasing Hormone and Adrenocorticotropin Secretion in Cushing's Disease and Major Depression: Potential Clinical Implications' cited in the application see the whole document	1-29
Y	WO,A,9 104 479 (THE UNITED STATES OF AMERICA; DEPT. OF COMMERCE) 4 April 1991	1-23
A	see the whole document	24-29
Y	WO,A,9 006 112 (THE UNITED STATES OF AMERICA; DEPT. OF COMMERCE) 14 June 1990	1-23
A	see the whole document	24-29
X	US,A,4 857 533 (FRED P. SHERMAN; DAVID C. ATKINSON) 15 August 1989	9,10,18
Y	see the whole document	1-23
	-/--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	THE AMERICAN JOURNAL OF PSYCHIATRY vol. 141, no. 5, May 1984, WASHINGTON DC US pages 619 - 627 PHILIP W. GOLD ET AL. 'Psychiatric Implications of Basic and Clinical Studies with Corticotropin Releasing Factor' cited in the application	24-29
A	see the whole document ---	1-23
Y	JOURNAL OF PSYCHIATRIC RESEARCH vol. 21, no. 3, March 1987, OXFORD GB pages 269 - 277 WILLIAM H. MELLER ET AL. 'Stimulation of the pituitary-adrenal axis with arginine vasopressin in patients with depression' cited in the application	24-29
A	see the whole document -----	1-23

INTERNATIONAL SEARCH REPORT

national application No.

PCT/US 93/04070

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-8, 18-25, and 28, 29 are directed to a diagnostic method and a therapeutical treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9304070
SA 74084

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

31/08/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9104479	04-04-91	US-A- 5209920	11-05-93
		AU-A- 6537490	18-04-91
		CA-A- 2067123	26-03-91
		EP-A- 0494256	15-07-92
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WO-A-9006112	14-06-90	US-A- 5006330	09-04-91
		AU-B- 621705	19-03-92
		AU-A- 4803990	26-06-90
		CA-A- 2004133	31-05-90
		EP-A- 0446282	18-09-91
		JP-T- 3503803	22-08-91
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US-A-4857533	15-08-89	AU-B- 617494	28-11-91
		AU-A- 3827989	21-06-90
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		JP-A- 2184627	19-07-90